

AIRBORNE MICROORGANISMS IN SHOW CAVES FROM ROMANIA

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Mesophilic bacteria and fungi were investigated in the air of three show caves in the Western Carpathians, Romania. The concentration of total aerobic viable bacteria, staphylococci, streptococci and Gram negative bacteria, and *Candida* spp. was generally higher within the visited areas, after the tourists' passage, than in the inner part of the caves or outside. In contrast, the dominant fungi *Aspergillus*, *Penicillium* and *Mucor* were found at higher concentration outside the caves, their number decreasing from the entrance towards the interior of the cave.

Key words: airborne microorganisms, mesophilic bacteria, fungi, show caves.

1. INTRODUCTION

The caves represent an extreme environment, characterized by specific ecological conditions, such as the absence of sunlight, a relatively constant temperature, high humidity, and slow airflows. The cave air is very low on dust, pollutants or bacteria, yeasts and aggressive allergens. Because of these qualities, the karstic aerosols were used in speleotherapy for about fifty years in some Central Europe countries, in the Balkans and in Turkey (GERJEVIĆ, 2002; KARAKOCA *et al.*, 1995). The microbiological studies concerning airborne microorganisms in caves are very scarce. MOLNAR (1961) performed such a study in the Baradla Cave, showing that *Bacillus megaterium* and *Sarcina* spp. were the most frequent airborne microorganisms, but the concentration of pathogenic bacteria was very low. In Romania, the microbial life of caves was studied in black sediments, moonmilk, clay and water (MANOLACHE, 2001), but not in the air.

The aim of the present study is to assess airborne microorganisms and the human impact in three show caves from Romania, in order to provide information about the air quality in this environment.

2. MATERIALS AND METHODS

STUDY LOCATION

The *Urșilor* Cave is located in the Crăiasa Valley, where the western part of the Bihor Mountains flows into the Beiuș depression. The cave is developed on two

levels. The fossil level, 800 m in length, was set up for public, according to the criteria of modern tourism, shortly after its discovery. The 700 m long active level is maintained as scientific reservation and is not open to visitors. In the year after its show opening in July 1980, the *Urşilor* Cave was visited by 283,727 people, presently accommodating over 100,000 tourists every year (FEKETE *et al.*, 1997).

The *Ungurului* Cave is located on the left side of the Crişul Repede river, upstream from its junction with the Mişidului Valley, in the northern part of the Pădurea Craiului Mountains. The total cave development attains 554 m, with an extension of 170 m and a ramification index of 3.2. It is an active cave, with a large entrance (33/20m) (VĂLENAŞ and IURKIEWICZ 1980–1981). The *Ungurului* cave was electrified and opened to the public in 2001, an average of 4,500 tourists' annual visiting the cave (I. Laţcu, pers. comm).

The *Vântului* Cave is the largest cave in Romania, with an extension of 3.5 km and a very high ramification index, a total length of 54 km being recorded up to now. The cave is located on the left slope of the Crişul Repede Valley, in the proximity of the *Ungurului* Cave. The *Vântului* Cave has four levels: the lowermost is active, being swept by a rivulet, while the other three are fossil. Certain areas of the first two levels received a minimal and ecological touristic setup, with no electric equipment installed, and are open to the public since 2003. The number of visitors is less than 2,500 per year, mainly during summer-time weekends.

SAMPLE COLLECTING

Air samples were collected before and after visitor passage, at two different locations for each cave. These locations were in the show area and in the scientific reservation for the first two caves. For the *Vântului* Cave, samples were collected in the active gallery, close to the entrance, and in the first fossil level (the "In memoriam" regrouping point). The exposure time was 10 min in show areas and 15 min in the scientific areas of the caves, respectively outside. The collected samples were kept at 4°C and were taken to the lab without delay.

CULTURE MEDIUMS

Airborne microorganisms were inoculated on agar media through gravitational fall-out of the microorganisms attached to solid particles in the air (Koch's sedimentation method). Five sterile media were used for the following groups of microorganisms:

1. Beef-extract agar medium – for the total count of aerobic bacteria;
2. Levine medium – for gram-negative bacteria;
3. Chapmann medium – for staphylococci;

4. Holmes medium – for streptococci;
5. Sabouraud medium – for fungi.

Media for the mesophilic microorganisms, as are total aerobic bacteria (TAG), gram-negative bacteria (GNB), staphylococci (SPH), and streptococci (STP), were incubated at 37°C for 24 hours. The medium for fungi (F) was incubated at 20–24°C for 3–5 days. The number of colony-forming units was calculated by means of Omelianski's formula and expressed per m³ of air (cfu/m³) (DRĂGHICI *et al.*, 2002). The identification of the fungi genera was made in accordance with the colonies' appearance (COMAN and MAREȘ, 2000).

3. RESULTS

This study is the first one to be carried out in show caves in Romania. Microbiological analyses were performed in three show caves with different visiting degrees: highly visited (*Urșilor Cave*), frequently visited (*Ungurului Cave*) and sporadically visited (*Vântului Cave*), respectively. We found that airborne microorganisms were present both outside and within the caves.

In the show areas of the *Urșilor Cave*, values of the mesophilic microorganisms were very high compared to outside or to those in the scientific reservation, where the human access is severely restricted. There was an exception for GNB, as these microorganisms were not found in the cave. The concentration of airborne microorganisms detected after the passage of about 400 visitors was also higher than the concentration recorded in the morning, before the area opened to public. In contrast, the concentration of fungi was higher outside the cave, while inside the number of colonies decreased towards the inner part of the cave (Table 1). The mycological examination of the incubated plates revealed the presence of the following genera: *Aspergillus*, *Candida*, *Penicillium* and *Mucor* outside the cave, *Aspergillus* and *Candida* in the show areas, and *Aspergillus*, *Candida* and *Penicillium* in the scientific areas of the caves.

The microbiological analysis performed in the *Ungurului Cave* showed a reduced level or even the absence of human-associated microorganisms if no visitors were present, and an increased concentration shortly after show time. Except for the concentration of TAG determined in the absence of visitors, we generally noticed a diminution of viable microorganisms in the air from the scientific reservation as compared with show areas. The total number of fungi detected within the cave showed no direct relation with the presence of public, higher values being recorded in the absence of visitors. No fungal spores were found in the scientific areas (Table 2). Five individuals of *Rhinolophus* bats were observed during the sample collecting procedure in the unvisited part of the cave. A colony of about 50 bats usually hibernates in those areas.

Table 1

The air microorganisms in the *Urşilor* Cavecfu/m³, colony forming units; TAG = total number of aerobic germs; GNB = Gram negative bacteria; SPH = *Staphylococcus*; STP = *Streptococcus*

Samples location (distance from entrance)	TAG (cfu/m ³)	GNB (cfu/m ³)	SPH (cfu/m ³)	STP (cfu/m ³)	Fungi (cfu/m ³)
Before visitors					
Outside	52	52	0	52	3196
Show areas (314 m)	707	0	157	79	3065
Scientific reservation (554 m)	52	0	0	53	1782
After visitors					
Outside	314	–	0	–	1100
Show areas (314 m)	1021	–	157	–	314
Scientific reservation (554 m)	523	–	52	–	105

Table 2

The air microorganisms in the *Ungurului* Cave (for abbreviations see Table 1)

Samples location (distance from entrance)	TAG (cfu/m ³)	GNB (cfu/m ³)	SPH (cfu/m ³)	STP (cfu/m ³)	Fungi (cfu/m ³)
Before visitors					
Show areas (148 m)	105	0	0	2	786
Scientific reservation (276 m)	262	0	0	0	734
After visitors					
Show areas (148 m)	472	52	52	209	52
Scientific reservation (276 m)	52	52	209	0	52

In the *Vântului* Cave, the microbiological assessments showed a prevalence of aerobic microorganisms inside the cave as compared to outside. In the absence of visitors, TAG and SPH persisted in the fossil gallery much longer than in the active gallery, near the entrance, where the ventilation is better. After a group of 22 visitors left, the concentration of mesophilic microorganisms was found to be higher in the first section of the cave, while in the inner areas TAG, GNB and SPH were absent. Airborne fungal spores were found at higher levels inside the cave, near the entrance (Table 3), where 14,425 cfu/m³ of *Candida* and 7,947 cfu/m³ of *Aspergillus* were counted. Because the plates exposed outside only revealed the presence of *Aspergillus*, it can be assumed that *Aspergillus* spores inside the cave came from outside, while *Candida* was brought by humans. The minimal count of fungi (represented only by *Aspergillus*) was noticed in the inner area of the cave, in the fossil gallery.

Table 3

The air microorganisms in the *Vântului* Cave (for abbreviations see Table 1)

Samples location (distance from entrance)	TAG (cfu/m ³)	GNB (cfu/m ³)	SPH (cfu/m ³)	STP (cfu/m ³)	F (cfu/m ³)
Before visitors					
Active gallery (8 m)	56	0	0	134	176
Fossil gallery (400 m)	112	0	106	134	235
After visitors					
Outside	78	0	0	78	13204
Active gallery (8 m)	419	52	0	157	22373
Fossil gallery (400 m)	0	0	0	105	2410

4. DISCUSSIONS

In the cave, where the humidity attains very high levels – very often close to saturation – the microorganisms are ubiquitous (JENSEN *et al.*, 1998). Airborne microorganisms survive in this subterranean environment because the degree of cellular stress and the evaporation rate diminish as relative humidity increases and temperature decreases (MARTHI and LIGHTHART, 1990). On the other hand, at a relative humidity above 70%, the microbial aerosols attached to solid particles turn into sedimentary aerosols and their sedimentation speed increases (DRĂGHICI, 1982).

Some of the airborne microorganisms can accumulate in the caves, *e.g.* staphylococci and streptococci, as well as *Candida* and *Aspergillus*, and may cause health problems in immunodeficient or hypersensitive individuals exposed to this environment (ABDEL HAMEED, 2002). Therefore, bioaerosol samplings are a valuable tool for the assessment of environmental quality.

TAG represents the sum of aerobic saprotrophic bacteria. Comparing the three studied show caves, we found values of TAG which varied between 0 and 1,205 cfu/m³, with a maximum in the show area of the *Urşilor* Cave, after the visitors' passage (Fig. 1, Tables 1–3). At a time when the cave was closed to public, in the *Ungurului* Cave we found higher TAG concentrations in the scientific or inner areas. Several factors may contribute to this: a high relative humidity (99%), weak air flows, and the presence of bats. Previous researches undertaken in a cave inhabited by bats, showed a direct relationship between their presence and airborne microorganisms, the airborne TAG being more abundant (11,317 cfu/m³) in the proximity of the bat colonies (BORDA *et al.*, 2004). TAG concentrations recorded in caves are lower than those recorded in human environments. For example, airborne TAG ranged between 629–10,479 cfu/m³ within Cluj-Napoca city limits and between 314–3,406 cfu/m³ in the pastures at the periphery of the city (DRĂGHICI, 1982).

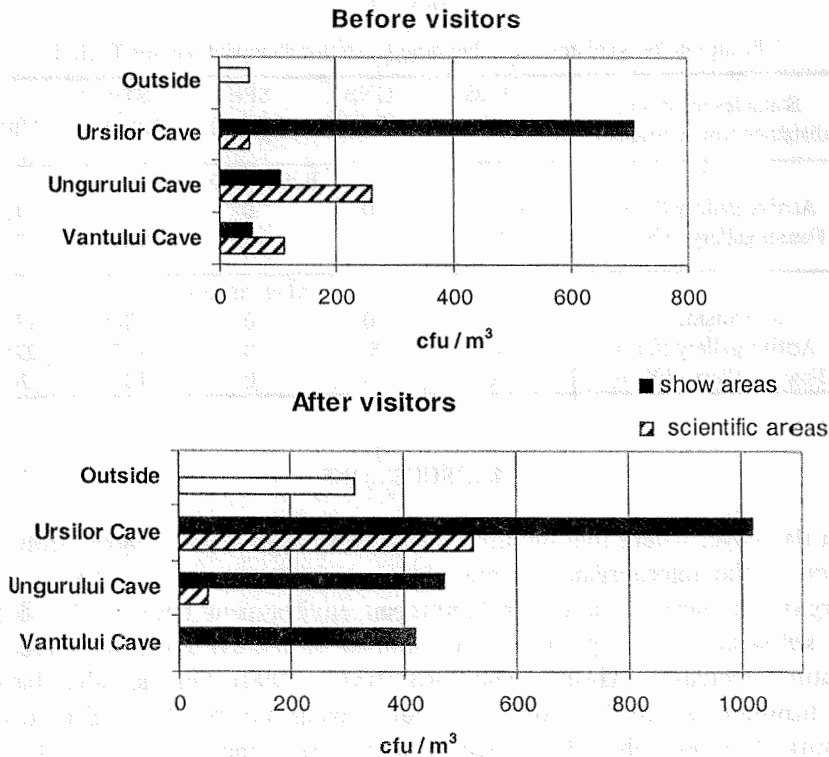


Fig. 1. – Total number of aerobic germs in the air of show caves.

GNB are characterized by a reduced content of polysaccharides and a high concentration of lipids in the wall cell. They do not originate in the cave, being brought in the *Vântului* Cave by visitors. SPH are Gram-positive bacteria, pathogenic or saprothrophic non-pathogenic, and STP are haemolytic bacteria. These genera were found everywhere in caves, both in show and in scientific areas, but they generally predominated in the visited areas and persisted after the tourists left. Gram-positive cocci may derive from airborne human skin scales and respiratory secretions containing such microorganisms (MOREY *et al.*, 1986). The presence of *Staphylococcus albus*-like organisms (VOLODIN and PSHENICHKOV, 1949) or *S. flavus* and *S. citr.* (MOLNAR, 1961) was also documented in cave air. Airborne *Staphylococcus capitis ureolyticus*, a chemoorganotrophic species, was isolated from the Lechuguilla Cave, possibly brought by humans (NORTHUP *et al.*, 1994; CUNNINGHAM *et al.*, 1995). It is also known that mesophilic viable bacteria, identified after being cultured at 37°C, are transient in caves, deriving from human or other mammalian sources (JENSEN and SCHAFER, 1998, LAVOIE *et al.*, 2000) and carried in by airflows or by animals and humans (MANOLACHE, 2001). Because of the scarce content of bacteria, especially pathogenic ones, in the cave

air in the absence of humans, MOLNAR (1961) proposed cave aerosols to be used for treatment of respiratory diseases. Showing that, with few exceptions, mesophilic microbial flora was more abundant in public areas, after the visitors' passage, our study also documents the influence of the presence of tourists.

Concerning the fungal flora, a high number and diversity was detected in the studied show caves. The concentration of fungi was higher near the entrance of the cave and decreased towards the deeper located scientific areas (Fig. 2). This fact

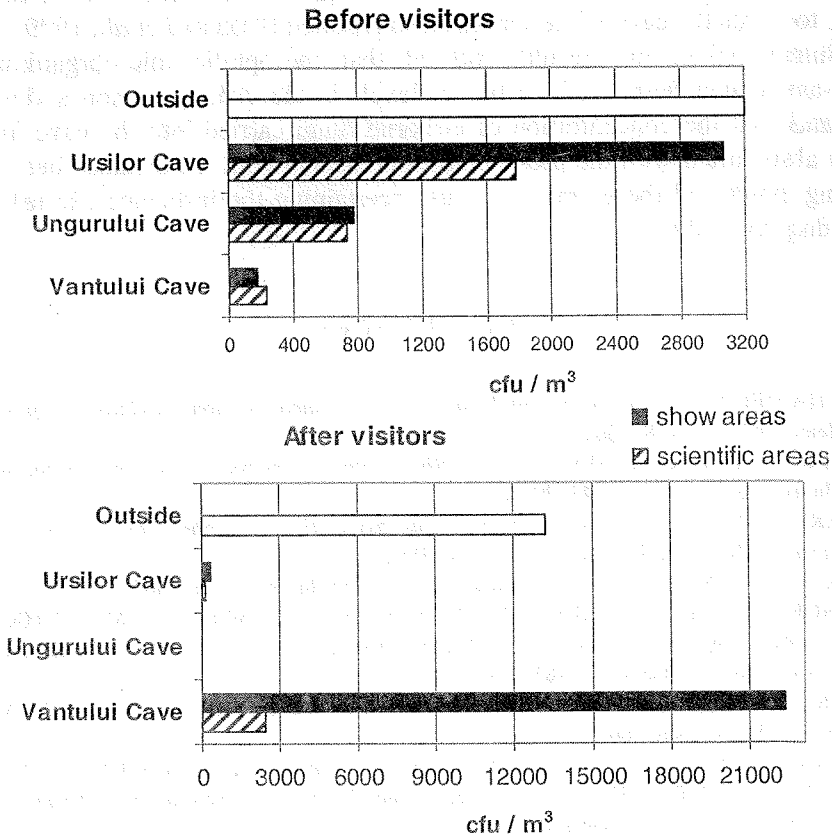


Fig. 2. – Fungi concentration in the air of show caves.

supports the assumption that fungi generally derive from the outside environment. Thus, the Bioaerosol Committee of AGGIH states that outdoor airborne fungi concentration routinely exceeds 1,000 cfu/m³ and may average 10,000 cfu/m³ in summer months (JENSEN and SCHAFER, 1998). *Aspergillus*, *Penicillium*, and sporadically *Mucor* genera, identified by us in the studied caves, are commonly found in caves. *Aspergillus* was the most frequent genus detected in the studied caves. *Aspergillus* usually arises from outdoors, being isolated from soil or vegetal

remains (COMAN and MAREȘ, 2000). The abundance of fungal colonies detected in the *Vântului* Cave near the entrance is due to the additional presence of *Candida*, a human-associated fungus. Data in the literature confirm the presence of a great diversity of fungal species in caves. They are found on organic sediments, on bat faeces and soil samples (SEMIKOLENNYKH, 1997) and are more extensive near the entrance than in the dark areas of caves (CAUMARTIN, 1966). The distribution of the predominantly occurring genera *Aspergillus* and *Penicillium* decreased from the entrance towards the interior, whereas an increase of other genera, including *Mucor*, towards the cave's interior was also reported (KOILRAJ *et al.*, 1999).

Summarizing, our results showed that mesophilic microorganisms and human-associated fungi attained higher levels in the visiting season and in show areas, and that the concentration of airborne fungi carried into the cave from the outside also varied with the presence of tourists and with the distance between the sampling point and the entrance – thus ascertaining the influence exerted by the public display of the studied caves.

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